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PROGRESS REPORT
June 1, 1992

"Bioorganic Models for Protein Ion Channels"
(Contract number N00014-90-C-0083, R&T CODE 4412065)

Principal Investigator: W. F. DeGrado

Contract Title: Bioorganic Models for Protein Ion Channels

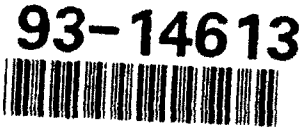
Start Date: April 1, 1990

Research objective: To investigate the structural features responsible for the conduction of ions through ion channel proteins; to design metal-binding peptides; to develop novel biosensing devices.

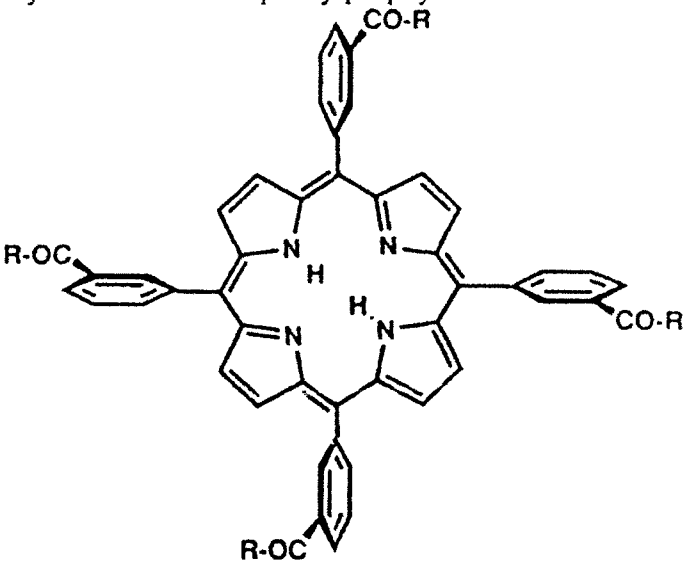
Progress (year 1):

Biophysical characterization of ion channels peptides We are using single-channel voltage clamp methods to study the model ion channel peptide, Ac-(LSSLLSL)3-$CONH_2$ in an attempt to determine how selective it is for the conductance of cations versus anions. Both ends of the peptide are masked with neutral blocking groups, allowing us to eliminate effects that are due to formal charges. Because the peptides can reorient upon a voltage change, we must use voltage pulses or fast ramps (approximately 100 msec) to measure full current-voltage relations on single open channels; the longer lifetime of the acetylated peptide facilitates this measurement. The open channel I-V relation rectifies in symmetrical 1 M KCl, with larger currents at the holding potential than at the opposite potential. (According to our gating model, the larger currents flow from C-terminus to N-terminus). To assess valence selectivity we apply a KCl gradient across the membrane, and measure the shift in the zero-current potential. Reducing KCl activity on the negative holding voltage (N-terminal) side by 2, 4, 8, and 16-fold shifts the zero-current by 14, 17, 9, and -14 mV, respectively. According to the Goldman-Hodgkin-Katz constant field theory, this indicates K/Cl permeability ratios of 10, 3.2, 1.6, and 0.53. This trend from cation to anion selectivity may arise from the electrostatic effect of the peptide helix dipole as the ionic strength decreases.

Template-Assembeled Channels Toward our goal of designing channel-forming helix-bundles of defined size we have continued to attempt to attach channel peptides to cyclodextrins. We have, however, encountered very difficult solubility problems with the



products, and the work is progressing slowly. On the other hand we have made considerable progress with peptides attached to phenylporphyrins (a collaboration with J Groves of Princeton). We have attached a proton channel peptide, (LSLBLSL)3, where B is alpha-aminoisobutyric acid onto tetraphenylporphyrin I.



R = -HN-(Leu-Ser-Leu-Aib-Leu-Ser-Leu)$_3$-CONH$_3$

The peptide assembly was purified using hydrophobic interaction chromatography and encorporated into small single bilayer vesicles. The CD spectrum of the peptide assembly indicated that the peptide had retained its helical character.

In planar bilayers, the porphyrin-peptide complex forms channels that are very similar to the parent peptide (LSLBLSL)$_3$ in conductance. However, the lifetime of the channels is considerably longer (1 - 2 msec), as compared to less than 0.05 msec for the parent (DeGrado and Lear; *Biopolymers*, **29**, 205-213 (1990)). Thus, the template has been successful in stabilizing the channel-forming state of the channel. In addition, channel formation is strongly voltage dependent for (LSLLLSL)$_3$, but not for the peptide/porphyrin assembly. A likely explanation of this finding is that (LSLLLSL)$_3$ binds to bilayers with its helical axis parallel to the bilayer surface whereas the covalently stabilized assembly of peptides forms a bundle that is vertically inserted in the membrane, even in the absence of a transmembrane electrical field.

De Novo Design of Metal Binding Sites in Proteins In previous work, we designed a Zn^{2+}-binding site in a water-soluble four-helix bundle protein by introducing two His residues on one helix and a third His on a linked helix, such that the ligating atoms could occupy three positions of a tetrahedron or iscosahedron (Handel, T. and DeGrado, W. F. *J. Amer. Chem. Soc.* **112**, 6710 (1990)). In the absence of metals, the protein shows an

NMR spectrum consistent with a "molten globule" structure, showing a high helical content but little dispersion in the resonances of the side chains. In addition, the protein binds the hydrophobic fluorescent probe ANS. Upon addition of metal ions, the protein becomes considerably more ordered as assessed by a dramatic increase in the dispersion of its NMR spectrum. In collaboration with Robin Hochstrasser (U. of Pennsylvania) we have examined the isotropic and polarized fluorescence decay of ANS bound to the protein. In the absence of bound zinc, the bound ANS shows substantial mobility within its binding site with a correlation time of 0.5 nsec. Upon the addition of zinc, its overall environment becomes highly restricted and its motion is determined only by the overall tumbling of the protein (correlation time 5 nsec). Also, the rates at which the amide protons exchange with solvent decreases by several orders of magnitude when zinc binds to the protein.

In an attempt to make the protein resemble native proteins even more closely, we have also changed the packing of the helix-helix interface to reduce the number of ways that the helices can pack. The resulting protein (Fig. 1) shows a transition from a native-like state at low temperature to a molten globule-like state at higher temperature with a midpoint near room temperature as assessed by NMR. We have also introduced a two-His, one-Glu metal binding site into this protein. This protein behaves like a native protein at all temperatures.

Significance The design of ion channel peptides should lead to a better understanding of of natural ion channel proteins, and may also lead to the development of elements to be used in biosensing devices. In addition, our design of a metal-binding protein should help decipher the mechanisms of cation selectivity in natural systems, and may be elaborated into sensing devices.

Work Plan (year 2): The objectives of year 2 are to: complete the synthesis of cyclodextrin-stabilized channels; further characterize the ion selectivity of the channels; characterize the conformational properties of metal-binding proteins.

Inventions (Year 2): None

Publications and Reports

1. Lovejoy, B., Åkerfeldt, K. S., DeGrado, W. F., Eisenberg, D." Crystallization of a Proton Channel Peptide" *Protein Science* in press.

2. Osterhaut, J., J., Jr., Handel, T., Na, G., Toumadje, A., Long, R. C., Connolly, P. J., Hoch, J. C. Johnson, W. C., Jr., Live, D., DeGrado, W. F. "Characterization of a Peptide Designed to Form a Four-Helix Bundle" *J. Amer. Chem. Soc.* **114**, 331-337 (1992).

3. Chung, L., Lear, J. D., and DeGrado, W. F. "Fluorescence Studies of the Secondary Structure and Orientation of a Model Ion Channel Peptide in Phospholipid Vesicles" *Biochem. in press*

4. Live, D., Osterhaut, J. J., Jr., Hoch, J. C., Handel, T. and DeGrado, W. F. in *Proceedings of the Twelfth American Peptide Symposium.*" (1991).

5. DeGrado, W. F., Raleigh, D. P., Handel, T. "De Novo protein design: what are we learning?" *Current Opinion in Structrual Biology* **1**, 984-993 (1991)

6. Lear, J. D., Wasserman, Z. R., DeGrado, W. F. "The use of synthetic peptides for the study of protein structure" in Membrane Protein Structure S. White, ed., Oxford University Press; in press.

7. Åkerfeldt, K. A., Lear, J. D., Wasserman, Z. R., Chung, L., DeGrado, W. F. "Synthetic peptide models for protein ion channels" Acc. Chem. Res. to be submitted.

8. Handel, T. M., Williams, S., DeGrado, W. F. "A Designed Protein, alpha4, shows characteristics of both the molten globule and native states" submitted to *Science*

Presentations (Invited lectures, April 1-June 30)

William F. DeGrado: Photosynthesis Gordon Conference, U. of Michigan, U. of Maryland, Haverford College, Fogarty Conference (NIH), ONR conference on Metal Ion Biosensors, Genentech, ACS Meeting, NIH.

Tracy Handel: Proteins Gordon Conference, Biophysical Society, ACS Meeting.

ANNUAL REPORT QUESTIONAIRE

Principal Investigator	William F. DeGrado
Institute	DuPont Merck Pharmaceutical Company Biotechnology Department
Grant title:	Bioorganic models for ion channel peptides
Period of performance	June 30, 1991 - June 1, 1992
Number of publications	8
Patents/inventions	
Number of trainees	3
female	1
Minority	0
Not U.S citizens	0
Awards, Honors	Eli Lilly Award for Biological Chemistry (ACS)
Equipment Purchased	None
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DE NOVO DESIGN OF

ION CHANNEL PEPTIDES & METAL ION BINDING PROTEINS

Objectives

- Design of template assembled channels
- Control selectivity of permeant ions
- Design of selective ion-binding proteins.
- Design of models for metalloproteinases and redox proteins.

Accomplishments

- Dynamics and structural properties of de novo designed Zn^{2+}-binding protein
- Design of models for blue copper proteins.
- Characterization of template-assembled channels.
- Modulation of channel lifetime.

Significance

- Learn to design proteins from scratch
- Learn how natural proteins work
- Design of Biosensors

α_2

HELIX: G E L E E L L K K L K E L L K G

P

R LOOP

R

HELIX: G K L L E K L K K L L E E L E G

MODIFIED α_2

HELIX: G E V E E L L K K F K E L W K G

P

R LOOP

R

HELIX: G K I L E K F K K F L E E I E G

MODIFIED α_2 METAL BINDING PEPTIDE

HELIX: G E V E E L E K K F K E L W K G

P

Zn^{2+} R LOOP

R

HELIX: G K I L E H F K K H L E E I E G

(fig. 1)